(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

B3

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 03/072014 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/16877

(22) International Filing Date: 28 May 2002 (28.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/359,843	25 February 2002 (25.02.2002)	US
10/154,951	24 May 2002 (24.05.2002)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	10/154,951 (CIP)
Filed on	24 May 2002 (24.05.2002)
US	60/359,843 (CIP)
Filed on	25 February 2002 (25.02.2002)

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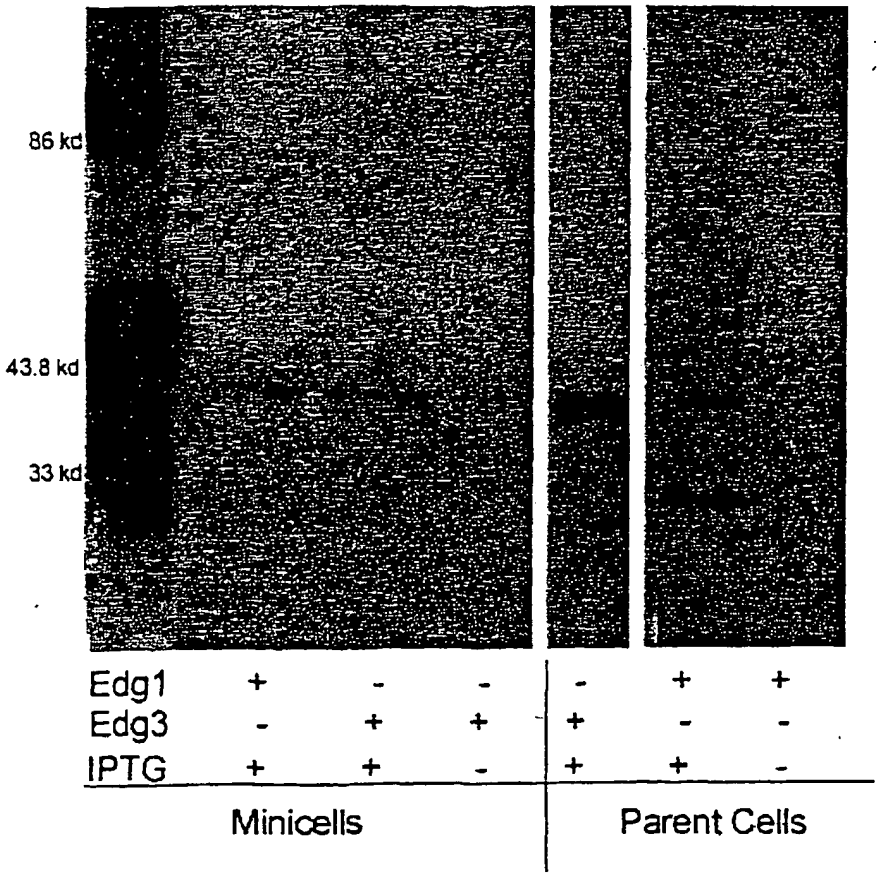
(54) Title: MINICELL COMPOSITIONS AND METHODS



(57) Abstract: The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

WO 03/072014 A2

(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

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CLAIMS

1. A minicell comprising a membrane protein selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

2. The minicell of claim 1, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

3. The minicell of claim 1, wherein said minicell comprises a biologically active compound.

4. The minicell of claim 1, wherein said minicell comprises a expression construct, wherein said first expression construct comprises expression sequences operably linked to an ORF that encodes a protein.

5. The minicell of claim 4, wherein said ORF encodes said membrane protein.

6. The minicell of claim 4, wherein said expression sequences that are operably linked to an ORF are inducible and/or repressible.

7. The minicell of claim 4, wherein said minicell comprises a second expression construct, wherein said second expression construct comprises expression sequences operably linked to a gene.

8. The minicell of claim 7, wherein said expression sequences that are operably linked to a gene are inducible and/or repressible.

9. The minicell of claim 7, wherein the gene product of said gene regulates the expression of the ORF that encodes said protein.

10. The minicell of claim 7, wherein the gene product of said gene is a nucleic acid.

11. The minicell of claim 7, wherein the gene product of said gene is a polypeptide.

12. The minicell of claim 11, wherein said polypeptide is a membrane protein, a soluble protein or a secreted protein.

13. The minicell of claim 12, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

14. A minicell comprising a membrane fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide.

15. The minicell of claim 14, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

16. The minicell of claim 14, wherein said minicell comprises a biologically active compound.

17. A minicell comprising a membrane conjugate, wherein said membrane conjugate comprises a membrane protein chemically linked to a conjugated compound.

18. The minicell of claim 17, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

19. The minicell of claim 17, wherein said minicell comprises a biologically active compound.

20. The minicell of claim 17, wherein said conjugated compound is selected from the group consisting of a nucleic acid, a polypeptide, a lipid and a small molecule.

21. A method for making minicells, comprising

(a) culturing a minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises a gene operably linked to expression sequences that are inducible and/or repressible, and wherein induction or repression of said gene causes or enhances the production of minicells; and

(b) separating said minicells from said parent cell, thereby generating a composition comprising minicells,

wherein an inducer or repressor is present within said parent cells during one or more steps and/or between two or more steps of said method.

22. The method of claim 21, further comprising

(c) purifying said minicells from said composition.

23. The method of claim 21, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

24. The method of claim 21, wherein said gene expresses a gene product that is a factor that is involved in or modulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

25. The method of claim 21, wherein said minicells are separated from said parent cells by a process selected from the group consisting of centrifugation, ultracentrifugation, density gradation, immunoaffinity and immunoprecipitation.

26. The method of claim 22, wherein said minicell is a poroplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that degrades the outer membrane of said minicell.

27. The method of claim 26, wherein said outer membrane is degraded by treatment with an agent selected from the group consisting of EDTA, EGTA, lactic acid, citric acid, gluconic acid, tartaric acid, polyethyleneimine, polycationic peptides, cationic leukocyte peptides, aminoglycosides, aminoglycosides, protamine, insect cecropins, reptilian magainins, polymers of basic amino acids, polymixin B, chloroform, nitrilotriacetic acid and sodium hexametaphosphate and/or by exposure to conditions selected from the group consisting of osmotic shock and insonation.

28. The method of claim 26, further comprising removing one or more contaminants from said composition.

29. The method of claim 28, wherein said contaminant is LPS or peptidoglycan.

30. The method of claim 29, wherein said LPS is removed by contacting said composition to an agent that binds or degrades LPS.

31. The method of claim 21, wherein said minicell-producing parent cell comprises a mutation in a gene required for lipopolysaccharide synthesis.

32. The method of claim 22, wherein said minicell is a spheroplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the outer membrane; and

(e) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the cell wall.

33. The method of claim 32, wherein said agent that disrupts or degrades the cell wall is a lysozyme, and said set of conditions that disrupts or degrades the cell wall is incubation in a hypertonic solution.

34. The method of claim 22, wherein said minicell is a protoplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupt or degrade the outer membrane;

(e) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the cell wall, in order to generate a composition that comprises protoplasts; and

(f) purifying protoplasts from said composition.

35. The method of claim 22, further comprising preparing a denuded minicell from said minicell.

36. The method of claim 22, further comprising covalently or non-covalently linking one or more components of said minicell to a conjugated moiety.

37. A method of preparing a L-form minicell comprising:

(a) culturing an L-form eubacterium, wherein said eubacterium comprises one or more of the following:

(i) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene regulates the copy number of an episomal expression construct;

(ii) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct.

(iii) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(iv) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

(b) culturing said L-form minicell-producing parent cell in media under conditions wherein minicells are produced; and

(c) separating said minicells from said parent cell, thereby generating a composition comprising L-form minicells,

wherein an inducer or repressor is present within said minicells during one or more steps and/or between two or more steps of said method.

38. The method of claim 37, further comprising

(d) purifying said L-form minicells from said composition.

39. A method of producing a protein, comprising:

(a) transforming a minicell-producing parent cell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said protein;

(b) culturing said minicell-producing parent cell under conditions wherein minicells are produced; and

(c) purifying minicells from said parent cell,

(d) purifying said protein from said minicells.

wherein said ORF is expressed during step (b), between steps (b) and (c), and during step (c).

40. The method of claim 39, wherein said expression elements segregate into said minicells, and said ORF is expressed between steps (c) and (d).

41. The method of claim 39, wherein said protein is a membrane protein.

42. The method of claim 39, wherein said protein is a soluble protein contained within said minicells, further comprising:

(e) at least partially lysing said minicells.

43. The method of claim 39, wherein said protein is a secreted protein, wherein said method further comprises

(e) collecting a composition in which said minicells are suspended or with which said minicells are in contact.

44. The method of claim 39, wherein the expression sequences to which said ORF is operably linked are inducible, wherein said method further comprises adding an inducing agent between steps (a) and (b); during step (b); and between steps (b) and (c).

45. The method of claim 39, wherein the expression sequences to which said ORF is operably linked are inducible, wherein said expression elements segregate into said minicells, said method further comprises adding an inducing agent after step (c).

46. The method of claim 39, further comprising:

(e) preparing poroplasts from said minicells,

wherein said ORF is expressed during step (b); between steps (b) and (c); during step (c); between step (c) and step (d) when said expression elements segregate into said minicells; and/or after step (d) when said expression elements segregate into said minicells.

47. The method of claim 46, further comprising:

(f) purifying said protein from said poroplasts.

48. The method of claim 39, further comprising

(e) preparing spheroplasts from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

49. The method of claim 48, further comprising:

(f) purifying said protein from said spheroplasts.

50. The method of claim 39, further comprising

(e) preparing protoplasts from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

51. The method of claim 50, further comprising:

(f) purifying said protein from said protoplasts.

52. The method of claim 39, further comprising

(e) preparing membrane preparations from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

53. The method of claim 48, further comprising:

(f) purifying said protein from said membrane preparations.

54. The method of claim 39, wherein said minicell-producing parent cell is an L-form bacterium.

55. A method of producing a protein, comprising:

(a) transforming a minicell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said protein; and

(b) incubating said minicells under conditions wherein said ORF is expressed.

56. The method of claim 55, further comprising:

(c) purifying said protein from said minicells.

57. The method of claim 55, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

58. A method of producing a protein, comprising:

(a) transforming a minicell-producing parent cell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes a fusion protein comprising said protein and a polypeptide, wherein a protease-sensitive amino acid sequence is positioned between said protein and said polypeptide;

(b) culturing said minicell-producing parent cell under conditions wherein minicells are produced;

(c) purifying minicells from said parent cell, wherein said ORF is expressed during step (b); between steps (b) and (c); and/or after step (c) when said expression elements segregate into said minicells; and

(d) treating said minicells with a protease that cleaves said sensitive amino acid sequence, thereby separating said protein from said polypeptide.

59. A poroplast, said poroplast comprising a vesicle, bonded by a membrane, wherein said membrane is an eubacterial inner membrane, wherein said vesicle is surrounded by a eubacterial cell wall, and wherein said eubacterial inner membrane is accessible to a compound in solution with said poroplast.

60. The poroplast of claim 59, wherein said poroplast is a cellular poroplast.

61. The poroplast of claim 59, wherein said compound has a molecular weight of at least 1 kD.

62. The poroplast of claim 59, wherein said poroplast comprises an exogenous nucleic acid.

63. The poroplast of claim 62, wherein said exogenous nucleic acid is an expression construct.

64. The poroplast of claim 63, wherein said expression construct comprises an ORF that encodes an exogenous protein, wherein said ORF is operably linked to expression sequences.

65. The poroplast of claim 64, wherein said poroplast comprises an exogenous protein.

66. The poroplast of claim 59, wherein said poroplast comprises an exogenous protein.

67. The poroplast of claim 66, wherein said exogenous protein is a fusion protein, a soluble protein or a secreted protein.

68. The poroplast of claim 66, wherein said exogenous protein is a membrane protein.

69. The poroplast of claim 68, wherein said membrane protein is accessible to compounds in solution with said poroplast.

70. The poroplast of claim 68, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein, and an organellar membrane protein.

71. The poroplast of claim 68, wherein said membrane protein is a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is displayed by said poroplast.

72. The poroplast of claim 71, wherein said second polypeptide is displayed on the external side of said eubacterial inner membrane.

73. The poroplast of claim 59, wherein said poroplast comprises a membrane component that is chemically linked to a conjugated compound.

74. The poroplast of claim 64, wherein said expression construct comprises one or more DNA fragments from a genome or cDNA.

75. The poroplast of claim 64, wherein said exogenous protein has a primary amino acid sequence that is predicted from in silico translation of a nucleic acid sequence.

76. A method of making poroplasts or cellular poroplasts, comprising treating eubacterial minicells or cells with an agent, or incubating said minicells or cells under a set of conditions, that degrades the outer membrane of said minicells or cells.

77. The method of claim 76, further comprising purifying said poroplasts or cellular poroplasts in order to remove contaminants.

78. The method of claim 76, further comprising placing said poroplasts in a hypertonic solution, wherein 90% or more of said cells or minicells used to prepare said poroplasts would lyse in said solution under the same conditions.

79. A solid support comprising a minicell.

80. The solid support of claim 79, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

81. The solid support of claim 79, wherein said solid support is a dipstick.

82. The solid support of claim 79, wherein said solid support is a bead.

83. The solid support of claim 79, wherein said solid support is a mictrotiter multiwell plate.

84. The solid support of claim 79, wherein said minicell comprises a detectable compound.

85. The solid support of claim 84, wherein said detectable compound is a fluorescent compound.

86. The solid support of claim 79, wherein said minicell displays a membrane component.

87. The solid support of claim 86, wherein said membrane component is selected from the group consisting of (i) a eukaryotic membrane protein, (ii) an archeabacterial membrane protein, (iii) an organellar membrane protein, (iv) a fusion protein comprising at least one transmembrane domain or at least one membrane anchoring domain, and (v) a membrane conjugate comprising a membrane component chemically linked to a conjugated compound.

88. The solid support of claim 86, wherein said membrane component is a receptor.

89. The solid support of claim 87, wherein said solid support further comprises a co-receptor.

90. The solid support of claim 79, wherein said minicell displays a binding moiety.

91. A solid support comprising a minicell, wherein said minicell displays a fusion protein, said fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide.

92. The solid support of claim 91, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

93. The solid support of claim 91, wherein said second polypeptide comprises a binding moiety.

94. The solid support of claim 91, wherein said second polypeptide comprises an enzyme moiety.

95. A solid support comprising a minicell, wherein said minicell comprises a membrane conjugate comprising a membrane component chemically linked to a conjugated compound.

96. The solid support of claim 95, wherein said conjugated compound is a spacer.

97. The solid support of claim 96, wherein said spacer is covalently linked to said solid support.

98. The solid support of claim 95, wherein said conjugated compound is covalently linked to said solid support.

99. A minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said binding moiety is part of a fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one

membrane anchoring domain and a second polypeptide that comprises a binding moiety, and said minicell is a poroplast, spheroplast or protoplast.

100. A eubacterial minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said binding moiety is selected from the group consisting of (a) a eukaryotic membrane protein; (b) an archeabacterial membrane protein; (c) an organellar membrane protein; and (d) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety.

101. The minicell of claim 99, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a receptor and an active site of a non-catalytic derivative of an enzyme.

102. The minicell of claim 99, wherein said binding moiety is a single-chain antibody.

103. The minicell of claim 99, wherein said binding moiety is directed to a ligand selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

104. The minicell of claim 99, wherein said biologically active compound is selected from the group consisting of a radioisotope, a polypeptide, a nucleic acid and a small molecule.

105. The minicell of claim 99, further comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

106. The minicell of claim 105, wherein one of said ORFs encodes a protein that comprises said binding moiety.

107. The minicell of claim 105, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

108. The minicell of claim 105, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

109. The minicell of claim 105, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

110. A method of associating a radioactive compound with a cell, wherein said cell displays a ligand specifically recognized by a binding moiety, comprising contacting said cell with a minicell that comprises said radioactive compound and displays said binding moiety.

111. The method of claim 110, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

112. The method of claim 110, wherein the amount of radiation emitted by said radioactive isotope is sufficient to be detectable.

113. The method of claim 110, wherein the amount of radiation emitted by said radioactive isotope is sufficient to be cytotoxic.

114. The method of claim 110, wherein said ligand displayed by said cell is selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

115. The method of claim 110, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a channel protein protein and a receptor.

116. The method of claim 110, wherein said binding moiety is a single-chain antibody.

117. The method of claim 110, wherein said binding moiety is selected from the group consisting of an aptamer and a small molecule.

118. A method of delivering a biologically active compound to a cell, wherein said cell displays a ligand specifically recognized by a binding moiety, comprising contacting said cell with a minicell that displays said binding moiety, wherein said minicell comprises said biologically active compound, and wherein the contents of said minicell are delivered into said cell from a minicell bound to said cell.

119. The method of claim 118, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

120. The method of claim 118, wherein said biologically active compound is selected from the group consisting of a nucleic acid, a lipid, a polypeptide, a radioactive compound, an ion and a small molecule.

121. The method of claim 118, wherein the membrane of said minicell comprises a system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell.

122. The method of claim 121, wherein said system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell is a Type III secretion system.

123. The method of claim 118, wherein said minicell further comprises a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

124. The method of claim 123, wherein one of said ORFs encodes a protein that comprises said binding moiety.

125. The method of claim 123, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

126. The method of claim 123, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

127. The method of claim 123, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

128. A minicell displaying a synthetic linking moiety, wherein said synthetic linking moiety is covalenty or non-covalently attached to a membrane component of said mincell.

129. The minicell of claim 128, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

130. A sterically stabilized minicell comprising a displayed moiety that has a longer half-life in vivo than a wild-type minicell, wherein said displayed moiety is a hydrophilic polymer that comprises a PEG moiety, a carboxylic group of a polyalkylene glycol or PEG stearate.

131. A minicell having a membrane comprising an exogenous lipid, wherein a minicell comprising said exogenous lipid has a longer half-life in vivo than a minicell lacking said exogenous lipid, and wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

132. The minicell of claim 131, wherein said exogenous lipid is a derivitized lipid.

133. The minicell of claim 132, wherein said derivitized lipid is selected from the group consisting of phosphatidylethanolamine derivatized with PEG, DSPE-PEG, PEG stearate; PEG-derivatized phospholipids, and PEG ceramides is DSPE-PEG.

134. The minicell of claim 131, wherein said exogenous lipid is not present in a wild-type membrane, or is present in a different proportion than is found in minicells comprising a wild-type membrane,

135. The minicell of claim 134, wherein said exogenous lipid is selected from the group consisting of ganglioside, sphingomyelin, monosialoganglioside GM1, galactocerebroside sulfate, 1,2-sn-dimyristoylphosphatidylcholine, phosphatidylinositol and cardiolipin.

136. The minicell of claim 128, wherein said linking moiety is non-covalently attached to said minicell.

137. The minicell of claim 136, wherein one of said linking moiety and said membrane component comprises biotin, and the other comprises avidin or streptavidin.

138. The minicell of claim 128, wherein said synthetic linking moiety is a cross-linker.

139. The minicell of claim 128, wherein said cross-linker is a bifunctional cross-linker.

140. A method of transferring a membrane protein from a minicell membrane to a biological membrane comprising contacting a minicell to said biological membrane, wherein said minicell membrane comprises said membrane protein, and allowing said mincell and said biological membrane to remain in contact for a period of time sufficient for said transfer to occur.

141. The method of claim 140, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

142. The method of claim 140, wherein biological membrane is a cytoplasmic membrane or an organellar membrane.

143. The method of claim 140, wherein said biological membrane is a membrane selected from the group consisting of a membrane of a pathogen, a membrane of an infected cell and a membrane of a hyperproliferative cell.

144. The method of claim 140, wherein said biological membrane is the cytoplasmic membrane of a recipient cell.

145. The method of claim 144, wherein said recipient cell is selected from the group consisting of a cultured cell and a cell within an organism.

146. The method of claim 140, wherein biological membrane is present on a cell that has been removed from an animal, said contacting occurs in vitro, after which said cell is returned to said organism.

147. The method of claim 144, wherein said membrane protein is an enzyme.

148. The method of claim 147, wherein said membrane protein having enzymatic activity is selected from the group consisting of a cytochrome P450 and a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one polypeptide, wherein said second polypeptide has enzymatic acitivity.

149. The method of claim 140, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

150. The method of claim 149, wherein said second polypeptide is a biologically active polypeptide.

151. The method of claim 140, wherein said minicell displays a binding moiety.

152. A minicell that comprises an expression construct comprising an ORF encoding a membrane protein operably linked to expression sequences, wherein said expression sequences are induced and/or derepressed when said minicell is in contact with a target cell.

153. The minicell of claim 152, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

154. The minicell of claim 152, wherein biological membrane is a cytoplasmic membrane or an organellar membrane.

155. The minicell of claim 152, wherein said biological membrane is a membrane selected from the group consisting of a membrane of a pathogen, a membrane of an infected cell and a membrane of a hyperproliferative cell.

156. The minicell of claim 152, wherein said minicell displays a binding moiety.

157. The minicell of claim 156, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, an aptamer and a small molecule.

158. The minicell of claim 152, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

159. The minicell of claim 152, wherein said membrane protein having enzymatic activity is selected from the group consisting of a cytochrome P450 and a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one polypeptide, wherein said second polypeptide has enzymatic activity.

160. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

161. The pharmaceutical composition of claim 160, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

162. The pharmaceutical composition of claim 160, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein, a cellular adhesion factor and an integrin.

163. The pharmaceutical formulation of claim 162, wherein said pharmaceutical formulation further comprises an adjuvant.

164. The pharmaceutical formulation of claim 162, wherein said membrane protein comprises a polypeptide epitope displayed by a hyperproliferative cell.

165. The pharmaceutical formulation of claim 162, wherein said membrane protein comprises an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus or an infected cell.

166. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein that is a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

167. The pharmaceutical composition of claim 166, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

168. The pharmaceutical formulation of claim 167, wherein said pharmaceutical formulation further comprises an adjuvant.

169. The pharmaceutical formulation of claim 167, wherein said second polypeptide comprises a polypeptide epitope displayed by a hyperproliferative cell.

170. The pharmaceutical formulation of claim 169, wherein said membrane protein comprises an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus or an infected cell.

171. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

172. The pharmaceutical composition of claim 171, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

173. The pharmaceutical composition of claim 171, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein, a cellular adhesion factor and an integrin.

174. The pharmaceutical composition of claim 171, wherein said pharmaceutical further comprises an adjuvant.

175. The pharmaceutical composition of claim 171, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

176. The pharmaceutical composition of claim 171, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

177. The pharmaceutical composition of claim 171, wherein said conjugated compound is a nucleic acid.

178. The pharmaceutical composition of claim 171, wherein said conjugated compound is an organic compound.

179. The pharmaceutical composition of claim 176, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, a sphingolipid and a soluble protein.

180. A method of making a pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

181. The method of claim 1, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

182. The method of claim 180, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

183. The method of claim 180, wherein said method further comprises desiccating said formulation.

184. The method of claim 183, wherein said method further comprises adding a suspension buffer to said formulation.

185. The method of claim 180, wherein said method further comprises making a chemical modification of said membrane protein.

186. The method of claim 185, wherein said chemical modification is selected from the group consisting of glycosylation, deglycosylation, phosphorylation, dephosphorylation and proteolysis.

187 A method of making a pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein that is a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second

polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

188. The method of claim 187, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

189. The method of claim 187, wherein said method further comprises desiccating said pharmaceutical formulation.

190. The method of claim 189 wherein said method further comprises adding a suspension buffer to said pharmaceutical formulation.

191. The method of claim 187, wherein said method further comprises making a chemical modification of said membrane protein.

192. The method of claim 191, wherein said chemical modification is selected from the group consisting of glycosylation, deglycosylation, phosphorylation, dephosphorylation and proteolysis.

193. A method of making a pharmaceutical formulation comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

194. The method of claim 193, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

195. The method of claim 193, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

196. The method of claim 193, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

197. The method of claim 193, wherein said conjugated compound is a nucleic acid.

198. The method of claim 193, wherein said conjugated compound is an organic compound.

199. The method of claim 186, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, and a sphingolipid.

200. A method of detecting an agent that is specifically bound by a binding moiety, comprising contacting a minicell displaying said binding moiety with a composition known or suspected to contain said agent, and detecting a signal that is modulated by the binding of said agent to said binding moiety.

201. The method of claim 200, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

202. The method of claim 200, wherein said agent is associated with a disease.

203. The method of claim 200, wherein said minicell comprises a detectable compound.

204. The method of claim 200, wherein said binding moiety is antibody or antibody derivative.

205. The method of claim 200, wherein said composition is an environmental sample.

206. The method of claim 200, wherein said composition is a biological sample.

207. The method of claim 206, wherein said biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, a biopsy sample, feces and a skin patch.

208. A method of in situ imaging of a tissue or organ, comprising administering to an organism a minicell comprising an imaging agent and a binding moiety and detecting said imaging agent in said organism.

209. The method of claim 208, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

210. The method of claim 208, wherein said binding moiety is an antibody or antibody derivative.

211. The method of claim 208, wherein said binding moiety specifically binds a cell surface antigen.

212. The method of claim 211, wherein said cell surface antigen is an antigen displayed by a tumorigenic cell, a cancer cell, and an infected cell.

213. The method of claim 211, wherein said cell surface antigen is a tissue-specific antigen.

214. The method of claim208, wherein said method of imaging is selected from the group consisting of magnetic resonance imaging, ultrasound imaging; and computer axaial tomography (CAT).

215. A device comprising a microchip operatively associated with a biosensor comprising a minicell, wherein said microchip comprises or contacts said minicell, and wherein said minicell displays a binding moiety.

216. The device of claim 215, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

217. A method of detecting a substance that is specifically bound by a binding moiety, comprising contacting the device of claim 215 with a composition known or suspected to contain said substance, and detecting a signal from said device, wherein said signal changes as a function of the amount of said substance present in said composition.

218. The method of claim 217, wherein said composition is a biological sample or an environmental sample.

219 A method of identifying an agent that specifically binds a target compound, comprising contacting a minicell displaying said target compound with a library of compounds, and identifying an agent in said library that binds said target compound.

220. The method of claim 219, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

221. The method of claim 219, wherein said library of compounds is a protein library.

222. The method of claim 221, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, a baculovirus library, a yeast display library, and a ribosomal display library.

223. The method of claim 219, wherein said library of compounds is selected from the group consisting of a library of aptamers, a library of synthetic peptides and a library of small molecules.

224. The method of claim 219, wherein said target compound is a target polypeptide.

225. The method of claim 224, wherein said minicell comprises an expression construct comprising expression sequences operably linked to an ORF encoding said target polypeptide.

226. The method of claim 224, wherein said target polypeptide is a membrane protein.

227. The method of claim 226, wherein said membrane protein is a receptor or a channel protein.

228. The method of claim 226, wherein said membrane protein is an enzyme.

229. The method of claim 219, wherein said target compound is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, wherein said first polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide comprises amino acid sequences derived from a target polypeptide.

230. The method of claim 219, wherein said method further comprises comparing the activity of said target compound in the presence of said agent to the activity of said target compound in the absence of said agent.

231. The method of claim 230, wherein said activity of said target compound is an enzyme activity.

232. The method of claim 231, wherein said enzyme is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

233. The method of claim 230, wherein said activity of said target compound is a binding activity.

234. The method of claim 233, further comprising comparing the binding of said agent to said target compound to the binding of a known ligand of said target compound.

235. The method of claim 234, wherein a competition assay is used for said comparing.

236. A device comprising microchips operatively associated with a biosensor comprising a set of minicells in a prearranged pattern, wherein said each of said microchips comprise or contact a minicell, wherein each of said minicell displays a different target compound, and wherein binding of a ligand to a target compound results in an increased or decreased signal.

237. A method of identifying an agent that specifically binds a target compound, comprising contacting the device of claim 236 with a library of compounds, and detecting a signal from said device, wherein said signal changes as a function of the binding of an agent to said target compound.

238. A method of identifying an agent that specifically blocks the binding of a target compound to its ligand, comprising contacting the device of claim 236 with a library of compounds, and detecting a signal from said device, wherein said signal changes as a function of the binding of an agent to said target compound.

239. A method of making a antibody that specifically binds a protein domain, wherein said domain is in its native conformation, wherein said domain is contained within a protein displayed on a minicell, comprising contacting said minicell with a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

240. The method of claim 239, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

241. The method of claim 239, wherein said protein displayed on a minicell is a membrane protein.

242. The method of claim 241, wherein said membrane protein is a receptor or a channel protein.

243. The method of claim 239, wherein said domain is found within the second polypeptide of a membrane fusion protein, wherein said membrane fusion protein comprises a first polypeptide, wherein said first polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain.

244. The method of claim 239, wherein said contacting occurs in vivo.

245. The method of claim 244, wherein said antibody is a polyclonal antibody or a monoclonal antibody.

246. The method of claim 244, wherein said contacting occurs in an animal that comprises an adjuvant.

247. The method of making an antibody derivative that specifically binds a protein domain, wherein said domain is in its native conformation, wherein said domain is displayed on a minicell, comprising contacting said minicell with a protein library, and identifying an antibody derivative from said protein library that specifically binds said protein domain.

248. The method of claim 247, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

249. The method of claim 247 wherein said antibody derivative is a single-chain antibody.

250. A method of making an antibody or antibody derivative that specifically binds an epitope, wherein said epitope is selected from the group consisting of (i) an epitope composed of amino acids found within a membrane protein, (ii) an epitope present in an interface between a membrane protein and a membrane component, (iii) an epitope present in an interface between a membrane protein and one or more other proteins and (iv) an epitope in a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, said second polypeptide comprising said epitope; comprising contacting a minicell displaying said epitope with a protein library, or to a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

251. The method of claim 250, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

252. The method of claim 250, wherein said cell is contacted in vivo.

253. The method of claim 252, wherein said antibody is a polyclonal antibody.

254. The method of claim 252, wherein said antibody is a monoclonal antibody.

255. The method of claim 250, wherein said protein library is contacted in vitro.

256. The method of claim 255, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

257. The method of claim 256, wherein said antibody derivative is a single-chain antibody.

258. A method of determining the rate of transfer of nucleic acid from a minicell to a cell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises said nucleic acid, for a set period of time;

(b) separating minicells from said cells;

(c) measuring the amount of nucleic acid in said cells,

wherein the amount of nucleic acid in said cells over said set period of time is the rate of transfer of a nucleic acid from a minicell.

259. A method of determining the amount of a nucleic acid transferred to a cell from a minicell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell; and

(b) detecting a signal from said detectable polypeptide,

wherein a change in said signal corresponds to an increase in the amount of a nucleic acid transferred to a cell.

260. The method of claim 258, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

261. The method of claim 258, wherein said cell is a eukaryotic cell.

262. The method of claim 258, wherein said binding moiety is an antibody or antibody derivative.

263. The method of claim 258, wherein said binding moiety is a single-chain antibody.

264. The method of claim 258, wherein said binding moiety is an aptamer.

265. The method of claim 258, wherein said binding moiety is an organic compound.

266. The method of claim 258, wherein said detectable polypeptide is a fluorescent polypeptide.

267. A method of detecting the expression of an expression element in a cell, comprising

(a) contacting said cell to a minicell, wherein said minicell comprises an expression element having cellular expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell; and

(c) detecting a signal from said detectable polypeptide,

wherein an increase in said signal corresponds to an increase in the expression of said expression element.

268. The method of claim 267, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

269. The method of claim 267, wherein said cell is a eukaryotic cell and said expression sequences are eukaryotic expression sequences.

270. The method of claim 269, wherein said eukaryotic cell is a mammalian cell.

271. The method of claim267, wherein said binding moiety is an antibody or antibody derivative.

272. The method of claim 267, wherein said binding moiety is a single-chain antibody.

273. The method of claim 267, wherein said binding moiety is an aptamer.

274. The method of claim 267, wherein said binding moiety is an organic compound.

275. The method of claim 267, wherein said detectable polypeptide is a fluorescent polypeptide.

276. A method for detecting the transfer of a fusion protein from the cytosol to an organelle of a eukaryotic cell, comprising

(a) contacting said cell to a minicell, wherein

(i) said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a fusion protein, wherein said fusion protein comprises a first polypeptide that comprises organellar delivery sequences, and a second polypeptide that comprises a detectable polypeptide; and

(ii) said minicell displays a binding moiety that binds an epitope of said cell, or an epitope of an organelle;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell and production of said fusion protein; and

(c) detecting a signal from the detectable polypeptide,

wherein a change in the signal corresponds to an increase in the amount of the fusion protein transferred to said organelle.

277. The method of claim 276, wherein said organelle is a mitochondrion, a chloroplast or a kinetoplast.

278. A minicell comprising at least one nucleic acid, wherein said minicell displays a binding moiety directed to a target compound, wherein said binding moiety is selected from the group consisting of (i) a eukaryotic membrane protein; (ii) an archeabacterial membrane protein; (iii) an organellar membrane protein; and (iv) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety.

279. The minicell of claim 278, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

280. The minicell of claim 278, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein, (ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

281. The minicell of claim 280, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

282. The minicell of claim 281, wherein said therapeutic polypeptide is a membrane polypeptide.

283. The minicell of claim 281, wherein said therapeutic polypeptide is a soluble polypeptide.

284. The minicell of claim 283, wherein said soluble polypeptide comprises a cellular secretion sequence.

285. The minicell of claim 281, wherein said expression sequences are inducible and/or repressible.

286. The minicell of claim2858, wherein said expression sequences are induced and/or derepressed when the binding moiety displayed by said minicell binds to its target compound.

287. The minicell of claim 1278herein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of a biologically active compound contained within or displayed by said minicell.

288 The minicell of claim 278 wherein the membrane of said minicell comprises a system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell.

289 The minicell of claim 288 wherein said system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell is a Type III secretion system.

290. A method of introducing a nucleic acid into a cell, comprising contacting said cell with a minicell that comprises said nucleic acid, wherein said minicell displays a binding moiety, wherein said binding moiety is selected from the group consisting of (i) a eukaryotic membrane protein; (ii) an archeabacterial membrane protein; (iii) an organellar membrane protein; and (iv) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety; and wherein said binding moiety binds an epitope of said cell.

291. The method of claim 290, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

292. The method of claim 290, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein,

(ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

293. The method of claim 290, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

294. The method of claim 293, wherein said expression sequences are inducible and/or derepressible.

295. The method of claim 294, wherein said expression sequences are induced or derepressed when the binding moiety displayed by said minicell binds its target compound.

296. The method of claim 294, wherein said expression sequences are induced or derepressed by a transactivation or transrepression event.

297. The method of claim 292, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of a biologically active compound contained within or displayed by said minicell.

298. A minicell comprising a nucleic acid, wherein said nucleic acid comprises eukaryotic expression sequences and eubacterial expression sequences, each of which is independently operably linked to an ORF.

299. The minicell of claim 298, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

300. The minicell of claim 298, wherein said minicell displays a binding moiety.

301. The minicell of claim 300, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

302. The minicell of claim 300, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

303. The minicell of claim 301, wherein the protein encoded by said ORF comprises eubacterial or eukaryotic secretion sequences.

304. A minicell comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a

second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

305. The minicell of claim 304, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

306. The minicell of claim 304, wherein said minicell displays a binding moiety.

307. The minicell of claim 306, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

308. The minicell of claim 306, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

309. The minicell of claim 304, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

310. A method of introducing into and expressing a nucleic acid in an organism, comprising contacting a minicell to a cell of said organism, wherein said minicell comprises said nucleic acid.

311. The method of claim 310, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

312. The method of claim 310, wherein said minicell displays a binding moiety.

313. The method of claim 310, wherein said nucleic acid comprises a eukaryotic expression construct, wherein said eukaryotic expression construct comprises eukaryotic expression sequences operably linked to an ORF.

314. The method of claim 310, wherein said ORF encodes a protein selected from the group consisting of a membrane protein, a soluble protein and a protein comprising eukaryotic secretion signal sequences.

315. The method of claim 310, wherein said nucleic acid comprises a eubacterial expression construct, wherein said eubacterial expression construct comprises eubacterial expression sequences operably linked to an ORF.

316. The method of claim 315, wherein said minicell displays a binding moiety, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

317. The method of claim 316, wherein the protein encoded by said ORF comprises eubacterial secretion sequences.

318. A minicell comprising a crystal of a membrane protein.

319. The minicell of claim 318, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

320. The minicell of claim 318, wherein said membrane protein is a receptor.

321. The minicell of claim 320, wherein said receptor is a G-protein coupled receptor.

322. The minicell of claim 318, wherein said crystal is displayed.

323. The minicell of claim 318, wherein said membrane protein is a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide.

324. The minicell of claim 323, wherein said crystal is a crystal of said second polypeptide.

325. The minicell of claim 323, wherein said crystal is displayed.

326. A method of determining the three-dimensional structure of a membrane protein, comprising preparing a crystal of said membrane protein in a minicell, and determining the three-dimensional structure of said crystal.

327. A method for identifying ligand-interacting atoms in a defined three-dimensional structure of a target protein, comprising (a) preparing one or more variant proteins of a target protein having a known or predicted three-dimensional structure, wherein said target protein binds a preselected ligand; (b) expressing and displaying a variant protein in a minicell; and (c) determining if a minicell displaying said variant protein binds said preselected ligand with increased or decreased affinity as compared to the binding of said preselected ligand to said target protein.

328. The method of claim 327, wherein said ligand is a protein that forms a multimer with said target protein, and said ligand interacting atoms are atoms in said defined three-dimensional structure are atoms that are involved in protein-protein interactions.

329. The method of claim 327, wherein said ligand is a compound that induces a conformational change in said target protein, and said defined three-dimensional structure is the site of said conformational change.

330. The method of claim 327, adopted to a method, said method for identifying ligands of a target protein, further comprising identifying the chemical differences in said variant proteins as compared to said target protein.

331. The method of claim 330, further comprising mapping said chemical differences onto said defined three-dimensional structure, and correlating the effect of said chemical differences on said defined three-dimensional structure.

332. The method of claim 331, wherein said target protein is a wild-type protein.

333. A minicell library, comprising two or more minicells, wherein each minicell comprises a different exogenous protein.

334. The minicell library of claim 333, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

335. The minicell library of claim 333, wherein said exogenous protein is a displayed protein.

336. The minicell library of claim 333, wherein said exogenous protein is a membrane protein.

337. The minicell library of claim 336, wherein said membrane protein is a receptor.

338. The minicell library of claim 333, wherein said protein is a soluble protein that is contained within or secreted from said minicell.

339. The minicell library of claim 333, wherein minicells within said library comprise an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said exogenous protein.

340. The minicell library of claim 339, wherein said nucleic acid has been mutagenized.

341. The minicell library of claim 339, wherein an active site of said exogenous protein has a known or predicted three-dimensional structure, and said a portion of said ORF encoding said active site has been mutagenized.

342. The minicell library of claim 333, wherein each of said minicells comprises an exogenous protein that is a variant of a protein having a known or predicted three-dimensional structure.

343. A minicell library, comprising two or more minicells, wherein each minicell comprises a different fusion protein, each of said fusion protein comprising a first

polypeptide that is a constant polypeptide, wherein said constant polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, wherein said second polypeptide is a variable amino acid sequence that is different in each fusion proteins.

344. The minicell library of claim 343, wherein minicells within said library comprise an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said fusion protein.

345. The minicell library of claim 344, wherein said second polypeptide of said fusion protein is encoded by a nucleic acid that has been cloned.

346. The minicell library of claim 344, wherein each of said second polypeptide of each of said fusion proteins comprises a variant of an amino acid sequence from a protein having a known or predicted three-dimensional structure.

347. A minicell library, comprising two or more minicells, wherein each minicell comprises a constant protein that is present in each minicell and a variable protein that differs from minicell to minicell.

348. The minicell library of claim 347, wherein one of said constant and variable proteins is a receptor, and the other of said constant and variable proteins is a co-receptor.

349. The minicell library of claim 347, wherein each of said constant and variable proteins is different from each other and is a factor in a signal transduction pathway.

350. The minicell library of claim 347, wherein one of said constant and variable proteins is a G-protein, and the other of said constant and variable proteins is a G-protein coupled receptor.

351. The minicell library of claim 347, wherein one of said constant and variable proteins comprises a first transrepression domain, and the other of said constant and variable comprises a second transrepression domain, wherein said transrepression domains limit or block expression of a reporter gene when said constant and variable proteins associate with each other.

352. The minicell library of claim 347, wherein one of said constant and variable proteins comprises a first transactivation domain, and the other of said constant and variable comprises a second transactivation domain, wherein said transactivation domains stimulate expression of a reporter gene when said constant and variable proteins associate with each other.

353. A method of identifying a nucleic acid that encodes a protein that binds to or chemically alters a preselected ligand, comprising:

(a) separately contacting said ligand with individual members of a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences, in order to generate a series of reaction mixes, each reaction mix comprising a different member of said minicell library;

(b) incubating said reaction mixes, thereby allowing a protein that binds to or chemically alters said preselected ligand to bind or chemically alter said preselected ligand;

(c) detecting a change in a signal from reaction mixes in which said ligand has been bound or chemically altered;

(d) preparing DNA from reaction mixes in which said ligand has been bound or chemically altered;

wherein said DNA is a nucleic acid that encodes a protein that binds to or chemically alters said preselected ligand.

354. The method of claim 353, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

355. The method of claim 353, wherein said preselected ligand is a biologically active compound.

356. The method of claim 353, wherein said preselected ligand is a therapeutic drug.

357. The method of claim 353, wherein a protein that binds or chemically alters said preselected ligand is a target protein for compounds that are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

358. The method of claim 353, wherein said preselected ligand is detectably labeled, said mincell comprises a detectable compound, and/or a chemically altered derivative of said protein is detectably labeled.

359. A method of determining the amino acid sequence of a protein that binds or chemically alters a preselected ligand, comprising:

(a) contacting said ligand with a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences;

(b) incubating said mixture of ligand and minicells, under conditions which allow complexes comprising ligands and minicells to form and/or chemical reactions to occur;

(c) isolating or identifying said complexes from said ligand and said mixture of ligand and minicells;

(d) preparing DNA from an expression element found in one or more of said complexes, or in a minicell thereof;

(e) determining the nucleotide sequence of said ORF in said DNA; and

(f) generating an amino sequence by in silico translation, wherein said amino acid sequence is or is derived from a protein that binds or chemically alters a preselected ligand.

360. The method of claim 359, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

361. The method of claim 359, wherein said DNA is prepared by isolating DNA from said complexes, or in a minicell thereof.

362. The method of claim 359, wherein said DNA is prepared by amplifying DNA from said complexes, or in a minicell thereof.

363. The method of claim 359, wherein said protein is a fusion protein.

364. The method of claim 359, wherein said protein is a membrane or a soluble protein.

365. The method of claim 364, wherein said protein comprises secretion sequences.

366. The method of claim 359, wherein said preselected ligand is a biologically active compound.

367. The method of claim 359, wherein said preselected ligand is a therapeutic drug.

368. The method of claim 359, wherein said preselected ligand is a therapeutic drug, and said protein that binds said preselected ligand is a target protein for compounds that

are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

369. A method of identifying a nucleic acid that encodes a protein that inhibits or blocks an agent from binding to or chemically altering a preselected ligand, comprising:

(a) separately contacting said ligand with individual members of a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences, in order to generate a series of reaction mixes, each reaction mix comprising a different member of said minicell library;

(b) incubating said reaction mixes, thereby allowing a protein that binds to or chemically alters said preselected ligand to bind or chemically alter said preselected ligand;

(c) detecting a change in a signal from reaction mixes in which said ligand has been bound or chemically altered;

(d) preparing DNA from reaction mixes in which said change in signal ligand has been bound or chemically altered;

wherein said DNA is a nucleic acid that encodes a protein that inhibits or blocks said agent from binding to or chemically altering said preselected ligand

370. The method of claim 369, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

371. The method of claim 369, wherein said DNA has a nucleotide sequence that encodes the amino acid sequence of said protein that inhibits or blocks said agent from binding to or chemically altering said preselected ligand.

372. The method of claim 369, wherein a protein that binds or chemically alters said preselected ligand is a target protein for compounds that are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

373. A method of identifying an agent that effects the activity of a protein, comprising contacting a library of two or more candidate agents with a minicell comprising said protein or a polypeptide derived from said protein, assaying the effect of candidate

agents on the activity of said protein, and identifying agents that effect the activity of said protein.

374. The method of claim 373, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

375. The method of claim 373, wherein said protein or said polypeptide derived from said protein is displayed on the surface of said minicell.

376. The method of claim 373, wherein said protein is a membrane protein.

377. The method of claim 376, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein and an enzyme.

378. The method of claim 373, wherein said activity of a protein is a binding activity or an enzymatic activity.

379. The method of claim 373, wherein said library of compounds is a protein library.

380. The method of claim 379, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

381. The method of claim 373, wherein said library of compounds is a library of aptamers.

382. The method of claim 373, wherein said library of compounds is a library of small molecules.

383. A method of identifying an agent that effects the activity of a protein domain containing a library of two or more candidate agents with a minicell displaying a membrane fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, wherein said second polypeptide comprises said protein domain.

384. A method of identifying undesirable side-effects of a biologically active compound that occur as a result of binding of said compound to a protein, wherein binding a compound to said protein is known to result in undesirable side effects, comprising contacting a minicell that comprises said protein to said biologically active compound.

385. The method of claim 384, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

386. The method of claim 384, further comprising characterizing the binding of said biologically active compound to said protein.

387. The method of claim 384, further comprising characterizing the effect of said biologically active compound on the activity of said protein.

388. A method for identifying an agent that effects the interaction of a first signaling protein with a second signaling protein, comprising

(a) contacting a library of compounds with a minicell, wherein said minicell comprises:

(i) a first protein comprising said first signaling protein and a first trans-acting regulatory domain;

(ii) a second protein comprising said second signaling protein and a second trans-acting regulatory domain; and

(iii) a reporter gene, the expression of which is modulated by the interaction between said first trans-acting regulatory domain and said second trans-acting regulatory domain; and

(b) detecting the gene product of said reporter gene.

389. The method of claim 388, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

390. The method of claim 388, wherein said trans-acting regulatory domains are transactivation domains.

391. The method of claim 388, wherein said trans-acting regulatory domains are transrepression domains.

392. The method of claim 388, wherein said reporter gene is induced by the interaction of said first trans-acting regulatory domain and said second trans-acting regulatory domain.

393. The method of claim 388, wherein said agent that effects the interaction of said first signaling protein with said second signaling protein is an agent that causes or promotes said interaction.

394. The method of claim 388, wherein said reporter gene is repressed by the interaction of said first trans-acting regulatory domain and said second trans-acting regulatory domain.

395. The method of claim 394, wherein said agent that effects the interaction of said first signaling protein with said second signaling protein is an agent that inhibits or blocks said interaction.

396. The method of claim 388, wherein said first signaling protein is a GPCR.

397. The method of claim 396, wherein said GPCR is an Edg receptor or a ScAMPER.

398. The method of claim 396, wherein said second signalling protein is a G-protein..

399. The method of claim 398, wherein said G-protein is selected from the group consisting of G-alpha-i, G-alpha-s, G-alpha-q, G-alpha-12/13 and Go.

400. The method of claim 388, wherein said library of compounds is a protein library.

401. The method of claim 400, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

402. The method of claim 388, wherein said library of compounds is a library of aptamers.

403. The method of claim 388, wherein said library of compounds is a library of small molecules.

404. A method for identifying an agent that effects the interaction of a first signaling protein with a second signaling protein, comprising contacting a library of two or more candidate agents with a minicell, wherein said minicell comprises:

(a) a first fusion protein comprising said first signaling protein and a first detectable domain; and

(b) a second fusion protein comprising said second signaling protein and a second detectable domain,

wherein a signal is generated when said first and second signaling proteins are in close proximity to each other, and detecting said signal.

405. The method of claim 404, wherein said signal is fluorescence.

406. The method of claim 404, wherein said first detectable domain and said second detectable domain are fluorescent and said signal is generated by FRET.

407. The method of claim 406, wherein said first and second detectable domains are independently selected from the group consisting of a green fluorescent protein, a blue-shifted green fluorescent protein, a cyan-shifted green fluorescent protein; a red-shifted green fluorescent protein; a yellow-shifted green fluorescent protein, and a red fluorescent protein, wherein said first fluorescent domain and said second fluorescent domain are not identical.

408. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said minicell alters the chemical structure and/or binds said undesirable substance.

409. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said mincell comprises an agent that alters the chemical structure of said undesirable substance.

410. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is an inorganic catalyst.

411. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is an enzyme.

412. The method of claim 411, wherein said enzyme is a soluble protein contained within said minicell.

413. The method of claim 412, wherein said soluble protein is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

414. The method of claim 411, wherein said enzyme is a secreted protein.

415. The method of claim 414, wherein said secreted protein is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

416. The method of claim 411, wherein said enzyme is a membrane protein.

417. The method of claim 416, wherein said membrane enzyme is selected from the group consisting of a cytochrome P450, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

418. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is a fusion protein comprising a first polypeptide that comprises a transmembrane domain or at least one membrane-anchoring domain, and a second polypeptide, wherein said second polypeptide is an enzyme moiety.

419. The method of claim 418, wherein said second polypeptide is a polypeptide derived from a protein selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

420. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said mincell comprises an agent that binds an undesirable substance.

421. The method of claim 420, wherein said undesirable substance binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

422. The method of claim 420, wherein said agent that binds said undesirable substance is a secreted soluble protein.

423. The method of claim 422, wherein said secreted protein is a transport accessory protein.

424. The method of claim 420, wherein said agent that binds said undesirable substance is a membrane protein.

425. The method of claim 420, wherein said undesirable substance is selected from the group consisting of a toxin, a pollutant and a pathogen.

426. The method of claim 420, wherein said agent that binds said undesirable substance is a fusion protein comprising a first polypeptide that comprises a transmembrane domain or at least one membrane-anchoring domain, and a second polypeptide, wherein said second polypeptide is a binding moiety.

427. The method of claim 426, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, the active site of a non-enzymatically active mutant enzyme, a single-chain antibody and an aptamer.

428. A minicell-producing parent cell, wherein said parent cell comprises one or more of the following:

(a) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or

repression of said gene regulates the copy number of an episomal expression construct;

(b) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct;

(c) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(d) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

429. The minicell-producing parent cell of claim 428, further comprising an episomal expression construct.

430. The minicell-producing parent cell of claim 428, further comprising a chromosomal expression construct.

431. The minicell-producing parent cell of claim 429, wherein said expression sequences of said expression construct are inducible and/or repressible.

432. The minicell-producing parent cell of claim 428, wherein said minicell-producing parent cell comprises a biologically active compound.

433. The minicell of claim 428 wherein said gene that causes or enhances the production of minicells has a gene product that is involved in or regulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

434. A minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises expression sequences operably linked to an ORF that encodes a protein, and a regulatory expression element, wherein said regulatory expression element comprises expression sequences operably linked to a regulatory gene that encodes a factor that regulates the expression of said ORF.

435. The minicell-producing parent cell of claim 434, wherein said expression sequences of said expression construct are inducible and/or repressible.

436. The minicell-producing parent cell of claim 434, wherein said expression sequences of said regulatory expression construct are inducible and/or repressible.

437. The minicell-producing parent cell of claim 434, wherein one or more of said expression element or said regulatory expression element is located on a chromosome of said parent cell.

438. The minicell-producing parent cell of claim 434, wherein one or more of said expression element or said regulatory expression element is located on an episomal expression construct.

439. The minicell-producing parent cell of claim 438, wherein both of said expression element and said regulatory expression element are located on an episomal expression construct, and one or both of said expression element and said regulatory expression element segregates into minicells produced from said parent cell.

440. The minicell-producing parent cell of claim 434, wherein said minicell-producing parent cell comprises a biologically active compound.

441. The minicell-producing parent cell of claim 440, wherein said biologically active compound segregates into minicells produced from said parent cell.

442. The minicell-producing parent cell of claim 434, wherein said ORF encodes a membrane protein or a soluble protein.

443. The minicell-producing parent cell of claim 434, wherein said protein comprises secretion sequences.

444. The minicell-producing parent cell of claim 434, wherein the gene product of said gene regulates the expression of said ORF.

445. The minicell-producing parent cell of claim 444, wherein said gene product is a transcription factor.

446. The minicell-producing parent cell of claim 440, wherein said gene product is a RNA polymerase.

447. The minicell-producing parent cell of claim 446, wherein said parent cell is MC-T7.

448. A minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said minicell selectively absorbs and/or internalizes an undesirable compound, and said minicell is a poroplast, spheroplast or protoplast.

449. The minicell of claim 448, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a receptor and an active site of a non-catalytic derivative of an enzyme.

450. The minicell of claim 458, wherein said binding moiety is a single-chain antibody.

451. The minicell of claim 458, wherein said binding moiety is directed to a ligand selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

452. The minicell of claim 458, wherein said biologically active compound is selected from the group consisting of a radioisotope, a polypeptide, a nucleic acid and a small molecule.

453. The minicell of claim 448, wherein a ligand binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

454. A pharmaceutical composition comprising the minicell of claim 448.

455. A method of reducing the free concentration of a substance in a composition, wherein said substance displays a ligand specifically recognized by a binding moiety, comprising contacting said composition with a minicell that displays said binding moiety, wherein said binding moiety binds said substance, thereby reducing the free concentration of said substance in said composition.

456. The method of claim 455, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

457. The method of claim 455, wherein said substance is selected from the group consisting of a nucleic acid, a lipid, a polypeptide, a radioactive compound, an ion and a small molecule.

458. The method of claim 455, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a channel protein and a receptor.

459. The method of claim 455, wherein said composition is present in an environment.

460. The method of claim 459, wherein said environment is water, air or soil.

461. The method of claim455, wherein said composition is a biological sample from an organism.

462. The method of claim 461, wherein said biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, a biopsy sample, feces, tissue and a skin patch.

463. The method of claim 461, wherein said substance binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

464. The method of claim 463, wherein said biological sample is returned to said organism after being contacting to said minicell.

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